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An Integrated Assessment of Geochemical and Community Structure Determinants of Metal Reduction Rates in Subsurface Sediments

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Results To Date

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Summary of Results to Date: Our current research represents a joint effort between Oak Ridge National Laboratory (ORNL), Florida State University (FSU), and the University of Tennessee. ORNL will serve as the lead institution with Dr. A.V. Palumbo responsible for project coordination, integration, and deliverables. This project was initiated in November, 2004, in the Integrative Studies Element of the NABIR program. The overall goal of our project is to provide an improved understanding of the relationships between microbial community structure, geochemistry, and metal reduction rates. The research seeks to address the following questions: Is the metabolic diversity of the in situ microbial community sufficiently large and redundant that bioimmobilization of uranium will occur regardless of the type of electron donor added to the system? Are their donor specific effects that lead to enrichment of specific community members that then impose limits on the functional capabilities of the system? Will addition of humics change rates of uranium reduction without changing community structure? Can resource-ratio theory be used to understand changes in uranium reduction rates and community structure with respect to changing C:P ratios?

The primary objective of year 1 was the development and optimization of microcosm experiments designed to manipulate uranium reduction activity (Task 1). Under Task 2 in year 1, we proposed to further define experimental conditions for the perturbation of microbial community structure by manipulating electron donor, electron shuttle, and nutrient concentrations. Task 1 is now nearly complete as 3 microcosm experiments were completed from November, 2004, to the present. Task 2 will be initiated during late

August and should be completed on schedule by the end of 2005 or early 2006.

During the Task 1 bounding experiments, microcosms were amended with a range of electron donors to alter the geochemistry, metabolic diversity, and activity of community members. The laboratory microcosms contained sediments and groundwater from the FRC site. Sediments samples were homogenized under anaerobic conditions prior to use in the microcosms. Carbon substrate concentrations were adjusted to give equivalent electron donor potential. Triplicate microcosms were run for each treatment (pH, substrate type). Each microcosm used 20 g of sediment and 80 ml of groundwater. The pH was adjusted using sodium bicarbonate. Unamended controls were included in each experiment. The microcosms were kept in a glove bag during the experiments. Analytical measurements were made weekly on each microcosm.

Carbon sources used included acetate, lactate, pyruvate, methanol, ethanol, glycerol, and glucose. These carbon sources were chosen to target specific physiological groups of bacteria, namely, fermentors, iron-reducers, sulfate-reducers, methanogens, and gram positive anaerobes.

Nitrate and uranium reduction rates were determined at ORNL. Some geochemical and physical parameters were measured at the time of sampling the microcosms. Lipid analyses were performed at UT.

Based on results of first microcosm experiment, the concentration of electron donor was doubled for the second experiment. Experiment two consisted of three electron donors (methanol (40 mM), ethanol (20 mM), and glucose (10 mM)), control (no added substrate), and used archived sediments and fresh groundwater. At the end of the time course of incubations, the microcosms were sacrificed and subsampled for lipid analysis. Ethanol treatment resulted in the most rapid nitrate reduction and a shorter lag time in comparison to glucose and methanol treatments. The control and methanol treatments showed no U reduction.

Based on the phospholipid fatty acid (PLFA) analysis the biomass of the original soil had ~126 pmol/g. The control microcosms biomass showed a 2-3 times greater biomass that the t-0 timepoint. At the final time point, biomass estimates of the microcosm experiment were 160-440 pmol/g for control, 2800-14900 pmol/g for methanol, 5000-8000 pmol/g for ethanol, and 7800-9500 pmol/g for glucose. Thus, biomass with carbon addition increased 21 to 115 fold of the control microcosms.

From the PLFA profiles, the gram negative communities (represented by monounsaturates) as compared to the original soil increased during the experiment. The methanol treatment had the largest relative proportion of gram negative bacteria. Glucose treatments had higher terminally branched saturates indicative of increases in gram positive bacteria.

Using principal component and cluster analyses on the PLFA profiles microcosms within the same treatment (ethanol, glucose and methanol) were similar. One control sample

was consistently different than the other two controls. This difference may be due to the low biomass detected in that sample as compared to the other control microcosms. Microcosms showing U reduction consisted of the ethanol and glucose treatments clustered together with samples from the sample treatment at higher similarities. The ethanol and glucose treatment cluster was separate from the control and methanol microcosms cluster. Interestingly, the PLFA profiles can also provide an indication of physiological stress. Higher stress was indicated in the control and methanol treatments. Nutrition stress in the methanol treatment and the control treatments was indicated by the cyclopropyl to monounsaturated fatty acid ratio. There also appears to be a potential toxicity stress in the methanol treatment indicated by the trans/cis ratio of monounsaturated fatty acids.

In conclusion, the electron donors had effects that lead to enrichment of specific community members. Also the methanol treatment imposed limits on the functional capabilities of the communities. There is sufficient metabolic diversity to accommodate many different electron donors (but perhaps not all) capable of stimulating bioimmobilization of uranium. Further experiments will take place with glucose, ethanol, and methanol with much more detailed community analysis.

The community analysis portion of the proposed research is to begin during Task 2, upon completion of the bounding experiments (Task 1), and in the second half of year 1. We are on schedule and the microcosms designed for community analysis will be conducted this month (August, 2005). In the first half of year 1, the Kostka lab has prepared for Task 2 by: 1)conducting experiments to streamline the analysis of FRC subsurface sediment communities present in situ using T-RFLP targeted to 16S rRNA gene targets, and 2) initiating microbial community analysis with conventional cloning/ sequencing of 16S rRNA genes in order to calibrate and verify the community fingerprinting by T-RFLP once Task 2 commences this month.

The incorporation of T-RFLP into our analysis of FRC subsurface sediments has been challenged by the extraction and amplification of sufficient quantities of PCR-quality DNA from low biomass sediments exposed to a range of geochemical conditions. We are able to measure the quantity of DNA in our extracts using spectrophotometric techniques and sufficient quantities of template are present for conventional PCR and cloning/ sequencing in a range of FRC sediments. However, we observed that PCR amplicon concentrations when using the fluorescently labeled forward primer (necessary for T-RFLP fingerprinting) were reduced compared to amplicon concentrations obtained with the non-labeled primers. Therefore, we tested whether a secondary PCR reaction of ten cycles using a fluorescently labeled forward primer would “label” non-fluorescent amplicons and yield similar T-RFLP profiles to that of a single PCR reaction using a fluorescently labeled forward primer. Trials were run on non-FRC sediment that we were able to easily obtain sufficient PCR amplicon concentrations from a single PCR reaction of 30 cycles. The results showed very different T-RFLP profiles, so we are now experimenting with additional methods for increasing the amplicon concentrations. We tested two different precipitation methods for concentrating amplicons and determined that using ammonium acetate as opposed

to sodium acetate resulted in higher fluorescent signal retention after re-hydration. We further increased the fluorescent signal by experimenting with a number of primer vendors and eventually changing vendors from IDT to ABI. Therefore, the final step to fully utilize T-RFLP to analyze and compare the microbial communities at the FRC is to increase our concentration of DNA extracted. We have made progress with a number of improved extractions and these will be ready for the onset of Task 2. Further, while improving our yield of fluorescent amplicon from FRC sediment extracts, we have used standard DNA from higher biomass sediment samples to streamline the remainder of our T-RFLP protocols.

Note that the above community characterization experiments were conducted on FRC subsurface sediment samples in which the in situ microbial communities have been determined to contain 126 pmol/g of PLFA biomass by our collaborator Dr. Pfiffner. Given that microcosm sediment samples analyzed during Task 1 contained at least one order of magnitude higher PLFA biomass levels, we expect that our improved fingerprinting protocols will work well during Task 2.

Deliverables

Papers and Other Products Delivered: The following 10 presentations and other products were completed during the past year of 2004 to 2005.

ORNL and UT-

A.V. Palumbo. 2005. Co-chaired breakout session with F. Brockman, “How distinct are microbial communities at different field sites?”, DOE-NABIR Principal Investigator Meeting, Warrenton, VA, 18-20 April. A.V. Palumbo, C.C. Brandt, J.E. Kostka, and S.M. Pfiffner. 2005. An integrated assessment of geochemical and community structure determinants of metal reduction rates in subsurface sediments. DOE-NABIR Principal Investigator Meeting, Warrenton, VA, 18-20 April. A.V. Palumbo, S.M. Pfiffner, L. Fagan, M. McNeilly, S. Rishell, J.E. Kostka, and C.C. Brandt. 2005. Substrate and community structure determinants of uranium reduction rates in subsurface sediments. Third European Bioremediation Conference, Athens, Greece, July.

FSU- Heath Mills was recruited during the past year to work on this project as a postdoctoral associate. Dr. Mills received his Ph.D. in Microbiology from the Georgia Institute of Technology under the direction of Patty Sobecky. His research specializes in microbial community analysis and the elucidation of structure-function relationships in sediments. Denise Akob, a new Ph.D. student in the Kostka lab, will focus her dissertation research on microbial communities in FRC subsurface sediments. She has completed her first year of training and has worked extensively on microcosm sediments for this project. Tom Gihring, another beginning Ph.D. student in the Kostka lab, has been assisting Ms. Akob in the analysis of microcosm materials. FSU personnel have been heavily involved in NABIR research, as evidenced by their regular

attendance at FRC workshops and NABIR PI meetings.

Kostka, J.E. 2005. “Metal Reducing Microbial Communities in the Acidic Subsurface,” University of Tubingen, Tubingen, Germany, March. Kostka, J.E. 2005. Linking Biodiversity to the Assessment of Bioremediation Potential in the Subsurface at DOE Sites. Plenary talk, DOE-NABIR Principal Investigator Meeting, Warrenton, VA, 18-20 April, 2005. Kostka, J.E. 2005. Naming the stars: coupling phylogeny with function during biostimulation. Breakout talk, DOE-NABIR Principal Investigator Meeting, Warrenton, VA, 18-20 April, 2005. Mills, H., and J.E. Kostka. 2005. New approaches for addressing the metabolically-active members of subsurface microbial communities. Breakout talk, DOE-NABIR Principal Investigator Meeting, Warrenton, VA, 18-20 April, 2005. D. M. Akob, H. J. Mills, L. Edwards, D. L. Balkwill, J. E. Kostka. Metabolically-Active Microbial Communities in Acidic Uranium-Contaminated Subsurface Sediments. Poster, DOE-NABIR Principal Investigator Meeting, Warrenton, VA, 18-20 April, 2005. D. M. Akob, H. J. Mills, L. Edwards, J. E. Kostka. The “Active” Microbial Communities found in Acidic Uranium-Contaminated Subsurface Sediments. Poster. Annual Meeting of the American Society for Microbiology, Atlanta, GA, June, 2005. Kostka, J.E. 2005. The Global Significance of Metal Reduction and Metal-Reducing Bacteria. Invited talk. Korea Ocean Research and Development Insitute, Seoul, Korea, June 18th, 2005.